Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 34, No. 1, 1999
ISSN 0735-1097/99/$20.00
PII S0735-1097(99)00144-8

Effects of Folate Supplementation in Hyperhomocysteinemic Pigs

Pierre Ambrosi, MD,* Pierre H. Rolland, PHD,† Heidi Bodard, PHD,† André Barlatier, PHD,‡ Philippe Charpiot, PHD,‡ Gwladys Guisgand, BSC,† Alain Friggi, PHD,† Odette Ghiringhelli, BSC,† Gilbert Habib, MD,* Gilles Bouvenot, MD,§ Danielle Garçon, PHD,‡ Roger Luccioni, MD, FACC*

Marseille, France

OBJECTIVES The aim of this study was to evaluate the therapeutic effects of folic acid in the pig model of hyperhomocysteinemia.

BACKGROUND We have previously shown that pigs fed a methionine-rich diet develop hyperhomocysteinemia, arterial lesions and thrombotic events. Elevated homocysteine level is an independent risk factor for atherosclerosis that can be markedly lowered with daily folic acid administration. However, it is not known whether this treatment can prevent arterial lesions.

METHODS Three groups of pigs were studied: 8 control subjects received a standard diet; 8 received a methionine-rich diet for four months; 8 received a methionine-rich diet for 1 month and then the methionine-rich diet + 5 mg/day folic acid for 3 months. At month 4 after hemodynamic investigation, all the pigs were sacrificed.

RESULTS Control animals developed few usual vascular streaks. All the pigs fed a methionine-rich diet without folic acid treatment developed hyperhomocysteinemia (10.3 ± 1.3 μmol/liter at basal state, 18.2 ± 2.5 μmol/liter at one month and 14.6 ± 3.8 μmol/liter at four months), hemodynamic abnormalities and diffuse arterial lesions with smooth muscle cell hyperplasia, endothelial alterations and elastic lamina dislocation. In this group, one pig died of venous thromboembolism and one of myocardial infarction. The pigs fed a methionine-rich diet + folic acid displayed similar arterial lesions and two had thrombotic events (one myocardial infarction and one pulmonary embolism), despite normalization of homocysteine levels (10.9 ± 1.3 μmol/liter at basal state, 19.5 ± 2.5 μmol/liter at one month and 11.4 ± 3.8 μmol/liter at four months).

CONCLUSIONS In the pig model of hyperhomocysteinemia, 5 mg/day folic acid did not prevent arterial lesions or thrombotic events. (J Am Coll Cardiol 1999;34:274–9) © 1999 by the American College of Cardiology

Mild hyperhomocysteinemia is a recognized independent risk factor for coronary artery disease, peripheral vascular disease, cerebrovascular disease and venous thromboembolism (1–3). Several causes of elevated plasma homocysteine levels have been identified. The strongest predictor of plasma total homocysteine level is the serum folate level (4–8). This vitamin is involved in the remethylation of homocysteine into methionine. Low folate levels are frequent among elderly people, are mainly related to low dietary intake, and are associated with a plasma homocysteine level elevation of about 40% (9,10). For folate levels below the lower limit, Kang et al. (10) reported that 84% of patients have hyperhomocysteinemia. Recent works have shown that in folate-deficient patients, mild hyperhomocysteinemia may result from an interaction with the genetic polymorphism of the methylene tetrahydrofolate reductase, an enzyme involved in the remethylation of homocysteine (11,12).

The treatments known to decrease plasma homocysteine levels are based upon oral or intramuscular administration of folates, vitamin B-6 and vitamin B-12. In subjects with mild hyperhomocysteinemia, folic acid 0.65 to 15 mg/day can lower homocysteinemia of about 20% to 45%, even in the presence of normal serum vitamins levels or in subjects with genetically caused hyperhomocysteinemia (10,13–15). Moreover, this treatment can improve arterial endothelial function in hyperhomocysteinemic subjects (16). These findings could have nutritional policy implications such as flour and other cereal product fortification with folic acid (8). However, it is unknown whether or not folic acid can prevent atherosclerosis or vascular events in mild hyperhomocysteinemia (2). In the present work we evaluated the therapeutic effects of folic acid treatment on the thrombotic and vascular consequences of hyperhomocysteinemia in the pig model.

From the *Department of Cardiology, Hôpital de la Timone, Marseille; †INSERM CJF 94-01; ‡Laboratory of Biochemistry, Faculté de Pharmacie, Marseille; §Laboratory of Therapeutics, Faculté de Médecine, Marseille, France. This work was supported by a grant of Rhône-Poulenc Rorer, Paris, France.

Manuscript received March 4, 1998; revised manuscript received January 29, 1999, accepted March 15, 1999.

Abbreviations and Acronyms

M0	= basal state
M1	= month 1
M4	= month 4
C group	= control group
M group	= pigs receiving a methionine-rich diet
M+F group	= pigs receiving a methionine-rich diet+folates
D	= external arterial diameter
P	= blood pressure
Q	= blood flow

METHODS

Animals, diets and treatments. The animals were handled in accordance with the INSERM U-278 Animal Care and Use Committee, as previously described (17,18). At basal state (M0), twenty-four 4.5-month-old Pietrin pigs (body weight = 44 kg) received either the methionine-rich diet previously reported to induce hyperhomocysteinemia (hyperhomocysteinemic pigs, [M group and M + F groups], n = 16 pigs) or a standard diet (controls, [C group], n = 8 pigs). The size of each group was chosen to allow a comparison of vascular histology and hemodynamics and was compatible with pig breeding and housing.

Hyperhomocysteinemic pigs were fed the methionine-rich diet for four months. Methionine-rich diet consisted of purified, delipidated caseinates (30 g/100), potato starch (59 g/100), large bran cellulose (7 g/100), fish starch (2 g/100), vitamins, salts and hog nectar (2 g/100). The 500-g daily ration corresponded to 1850 kcal and contained 3.45 g methionine, 2 mg vitamin B-6, 20 μg vitamin B-12, and 220 μg folic acid. One month after the beginning of this diet, eight of these pigs were given folic acid 5 mg daily for the last three months (M+F group) and eight were not (M group). Folic acid (5 mg tablet) (Speciafoldine, Rhône-Poulenc Rorer, France) was inserted in the food.

The eight pigs of the control group (C) received for four months a standard diet consisting of soybean proteins (30 g/100), potato starch (59 g/100), bran cellulose (7 g/100), fish starch (2 g/100), vitamins, mineral salt and hog nectar (2 g/100) (breeding diet 127, UAR, Villemoison sur Orge, France). The 500-g daily dose corresponded to 1850 kcal and contained 1.28 g methionine, 2 mg vitamin B-6, 20 μg vitamin B-12, and 220 μg folic acid.

At four months (M4), following hemodynamic investigations, pigs were sacrificed for pathologic analysis.

Blood collection and serum biochemistry. Blood samples were drawn in the morning (i.e., 24 h after the last treatment and feeding). Serum standard biochemistry was determined at M0 and M4. Serum methionine and homocysteine levels were determined at M0, M1, and M4 following the previously described method (17). The samples of plasma were stored at −20°C, then analyzed after reduction with dithiothreitol and deproteinization with sulfosalicylic acid. Reduced plasma samples then were chromatographed on a cationic ion exchange resin Beckman AA 6 300 amino acid analyzer (Beckman Instruments, Gagny, France) with lithium citrate buffer as eluent solvent and ninhydrin as revealing agent.

Histologic analysis of the vasculature. Histologic changes were evaluated in the abdominal aorta (in a 1.5-cm-long segment of abdominal aorta located at mid-distance between the left renal artery and aortic trifurcation), in the left interventricular coronary artery (in a 0.5-cm-long segment immediately after the circumflex artery bifurcation from the left coronary trunk), in the renal arteries (in a 1-cm-long segment at 1.5 cm from the ostium), and in the common carotid bifurcation (in a 0.5-cm-long segment of the distal left common carotid artery centered on the internal and external carotid artery bifurcations). Immediately after sacrifice, the segments of pig arteries were carefully rinsed in ice-cold isotonic saline solution and fixed in formol for 18 h. Serial slides were obtained and alternately stained with hematoxylin-eosin-safranine for general observation, Masson trichrome for connective and nuclear compounds, and orcein for elastic tissue. Computerized morphodensitometric analysis of orcein-stained pathologic slides of abdominal aorta and left interventricular coronary artery was performed to evaluate the elastin content within the media and to give a quantitative characterization of elastic structure.

Arterial structures were investigated using a light video microscope (Axioplan, Zeiss, Jena, Germany) linked to a SAMBA 2005 automatic image analyzer (TITN-Alcatel, Grenoble, France). A 10× eyepiece and a 10× objective for the abdominal aorta, or a 20× objective for the left interventricular coronary artery allowed observation of the whole width of the media within a single image. After selection of a zone of interest, the image was digitized on a 640 × 480 pixel frame using a normalized 256-gray-level scale. The analysis was carried out on a manually defined standardized rectangular field, whose major axis was a radial segment and whose width was fixed at 200 μm for the abdominal aorta and 100 μm for the left interventricular coronary artery. Stained elastic elements were selected onto the image by interactively setting a gray-level threshold. The morphometric parameters (length and area) of each selected object were then automatically computed. Objects with an area of less than 10 mm^2 were excluded as unidentifiable. Assuming homogeneity at staining, the mean thickness of each object was calculated as proportional to its mean residual gray level after subtracting the background, and its volume was calculated from its area and mean thickness. Medial elastin concentration was evaluated as the volume density of the stained component within the media by dividing the summed volumes of detected objects by the volume of the analyzed portion of slide, as previously described (19).

Table 1. Biochemistry (Mean ± SD)

	C Group Basal	M Group Basal	M+F Group Basal	C Group 4.5 months	M Group 4.5 months	M+F Group 4.5 months
Protein (G/liter)	67.8 ± 15.6	64.7 ± 7.6	61.2 ± 6.9	65.8 ± 5.5	67.3 ± 3.7	67.7 ± 7.2
Urea (mmol/liter)	3.8 ± 0.1	4.4 ± 1.6	3.8 ± 0.9	4.0 ± 0.5	10.6 ± 2.6	10.3 ± 2.6
Creatinine (mmol/liter)	156.7 ± 8.7	156.3 ± 33.9	161.2 ± 17.7	155.4 ± 6.6	141.6 ± 24.3	131.3 ± 16.6
Glucose (mmol/liter)	6.6 ± 1.6	4.8 ± 1.8	4.8 ± 2.4	6.1 ± 1.6	4.3 ± 0.4	3.4 ± 0.8
Calcium (mmol/liter)	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Triglycerides (mmol/liter)	0.4 ± 0.2	0.6 ± 0.4	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
HDL cholesterol (mmol/liter)	0.4 ± 0.4	0.8 ± 0.3	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
LDL cholesterol (mmol/liter)	1.0 ± 0.2	1.0 ± 0.5	0.7 ± 1.5	1.0 ± 0.5	0.9 ± 0.1	0.9 ± 0.1
Cholesterol (mmol/liter)	1.8 ± 0.7	1.8 ± 0.8	1.6 ± 0.1	1.8 ± 0.2	1.7 ± 0.2	1.8 ± 0.2
ASAT (IU/liter)	28.8 ± 0.1	26.6 ± 6.2	25.6 ± 9.1	29.4 ± 6.7	51.3 ± 20.1	49.7 ± 23.3
ALAT (IU/liter)	49.1 ± 1.6	31.1 ± 10.9	51.8 ± 12.4	57.2 ± 25.1	60.2 ± 19.3	65.1 ± 14.1
Gamma GT (IU/liter)	44.2 ± 12.6	60.8 ± 29.2	64.1 ± 11.6	55.3 ± 13.7	67.2 ± 19.7	78.1 ± 15.1
Cysteine (μmol/liter)	95.4 ± 13.1	113.0 ± 17.1	103.1 ± 10.1	101.0 ± 5.3	73.2 ± 7.8	78.0 ± 14.0
Methionine (μmol/liter)	22.7 ± 1.3	30.1 ± 8.0	24.2 ± 6.2	23.4 ± 3.1	21.68 ± 4.6	31.7 ± 7.2
Homocysteine (μmol/liter)	10.9 ± 2.1	10.3 ± 1.3	10.8 ± 2.4	10.8 ± 1.6	14.6 ± 3.8	11.4 ± 2.9

Scanning electron microscopy was performed as previously described (20). Briefly, a segment of artery was removed and sectioned longitudinally in two halves. Each specimen was fixed with ice-cold 2.5% buffered glutaraldehyde for 1 h and then dehydrated in progressive acetone and dried by the critical-point method with acetone and liquid carbon dioxide. After coating with 30 nm gold coating layer, scanning electron microscopy was performed with a JEOL JSM 35 CF microscope.

Hemodynamics. Hemodynamics of iliac and renal arteries were determined invasively at M4 following a previously described method (17). Briefly, external arterial diameter (D) was determined with a periarterial probe consisting of two piezoelectric crystals. Blood pressure (P) was determined using a 5 PR 249 3F pressure-sensor mikrotip catheter (Millar, Houston, Texas). Blood flow (Q) was measured using a T 206 periarterial ultrasonic blood flow probe (Transonic System). Individual-average diameter, flow, and pressure were obtained after digitization from 20 cycles, whose synchronization was triggered by the R-wave of the electrocardiogram (ECG). From these data, we determined the average population of pulsatile flow-pressure plots, arterial compliance ($\Delta V/\Delta P$), impedance ($\Delta P/\Delta Q$), stiffness ($\Delta P/\Delta D$), Young's elastic modulus and midwall arterial stress.

Statistical analysis. Data are reported as mean ± SD. Statistical analysis was performed using GB-stat (Dynamic Microsystems). Morphometry was analyzed with one-way analysis of variance (ANOVA), the hemodynamics and biochemical analysis with ANOVA for repeated measures, and followed by a pairwise comparison in case of statistical significance using the Scheffé test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Vascular events. All animals remained healthy throughout the treatment period and survived until euthanasia in the C (control) group. In the M group (methionine-rich diet), one pig died from pathologically confirmed pulmonary embolism at 3.5 months and one died of myocardial infarction at four months at the time of surgery for hemodynamic study. In the M+F group, one animal died from pulmonary embolism with extensive thrombosis in inferior vena cava and right atrium at 3.5 months; one pig died from myocardial infarction at the time of surgery.

Biochemistry. At basal state and at M4, the three groups did not differ significantly for standard biochemistry (Table 1). Folic acid treatment did not modify plasma fibrinogen, International Normalized Ratio and hematologic parameters (white and red blood cell counts, mean globular volume, platelet count; data not shown).

At basal state, serum methionine and homocysteine levels were similar in the three groups. Homocysteine (M0: 10.9 ± 2.1 μmol/liter; M4: 10.8 ± 1.6 μmol/liter) did not change in the C group. In the M group, plasma homocysteine concentration was higher at M1 than at M0 (18.2 ± 2.5 vs. 10.3 ± 1.3 μmol/liter, $p < 0.05$) and remained high at M4 (14.6 ± 3.8, $p = 0.08$ vs. M0). In the M+F group, plasma homocysteine concentration was higher at M1 than at M0 (19.5 ± 2.5 vs. 10.9 ± 1.3 μmol/liter, $p < 0.05$) and returned to the basal level at M4 on folic acid treatment (11.4 ± 3.8 μmol/liter). Methionine levels did not change between M0 and M4 in the C and M groups, and significantly increased in the M+F group (M0: 24.3 ± 6.2, M4: 31.7 ± 7.2 μmol/liter, $p < 0.05$).

Histopathology of vessels. In control animals, left interventricular coronary arteries and to a lesser extent, abdom-

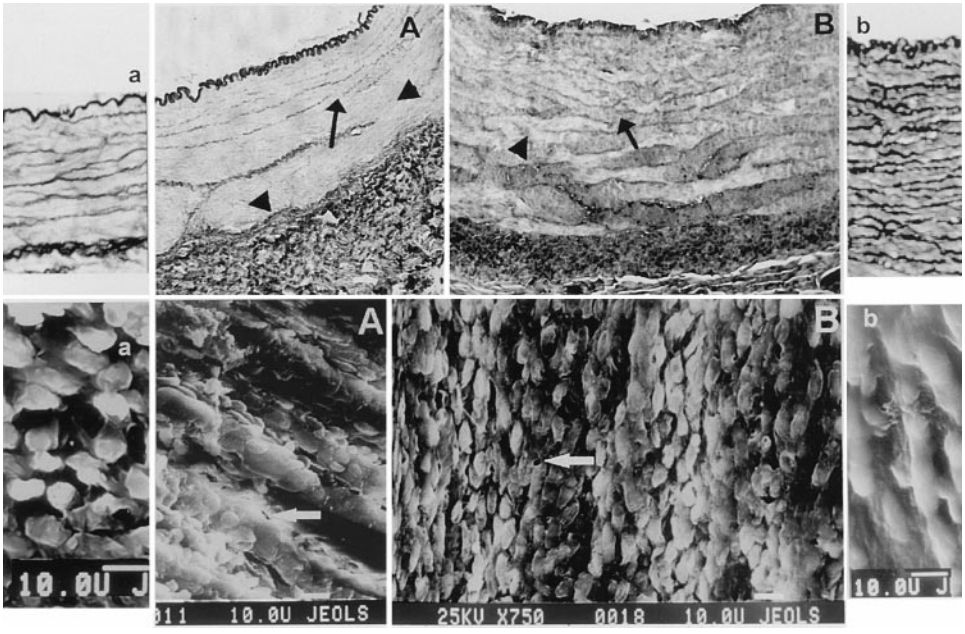

Figure 1. Photomicrographs of renal **(A,a)** and iliac **(B,b)** arteries of pigs fed the control **(small letters)** or the methionine-rich diet **(capitals)**. The transverse sections **(black letters)** were obtained at ×150 after orcein staining. They show elastic lamina fragmentation **(black arrow)** and cellular hyperplasia **(black arrowheads)** in the media. The views of the luminal surface of the endothelium **(white letters)** were obtained with scanning electron microscopy at ×750. Endothelial cell bulging in the arterial lumen is associated with intercellular holes **(white arrows)**.

inal aortas exhibited few vascular streaks. In the M and M+F groups, the abdominal aorta, carotid bifurcations, left interventricular coronary artery and renal arteries showed alterations of endothelial cells, elastic lamina disruption and smooth muscular cell hyperplasia, characteristics of the homocysteine-induced lesions in the vascular walls of hyperhomocysteinemic animals. The disorganization of elastic fibers was diffuse and present in all studied arteries. Aortic and carotid lesions were barely protruding in the vascular lumen. In the coronary and renal arteries, medial and intimal hyperplasia caused the lesion to bulge in the lumen. In the M and M+F groups, scanning electronic microscopy of the vascular luminal surface showed endothelial cells heterogeneous in shape, bulging in the vascular lumen, with sparse intercellular "holes" (Fig. 1).

The endothelial surface was smooth without intercellular holes in the C group. Medial thickness was similar in the three groups at histomorphometry (Table 2). Medial elastin concentration was lower in hyperhomocysteinemic animals than in the controls. Medial elastin concentration was similar in the M and M+F groups (Table 2).

Hemodynamics. Hemodynamic study was achieved in six pigs of each group (Table 3).

RENAL VERSUS ILIACS HEMODYNAMICS. There were no significant differences in blood pressure or mean flow between iliacs and renal arteries in the C group. Wall thickness-mean radius ratio and arterial stiffness were higher in renal than in iliac arteries. Arterial compliance and pulsatility were lower in renal arteries, indicating that the elastic component is prominent in the iliacs, in contrast with the renal arteries in which the viscous part is the major wall component.

EFFECT OF HYPERHOMOCYSTEINEMIA. Blood pressure was not modified in the M group versus the C group. In iliac arteries, a nonsignificant decrease in compliance and an in-

Table 2. Histomorphometric Analysis of the Abdominal Aorta and of the Left Interventricular Coronary Artery in the Three Groups

	Abdominal Aorta			Coronary Artery		
	C Group	M Group	M+F Group	C Group	M Group	M+F Group
Medial elastin concentration (volume %) ± SD	11.7 ± 0.9	9.6 ± 2.8*	9.0 ± 1.0*	9.9 ± 1.3	6.3 ± 1.4*	7.4 ± 1.4*
Medial thickness (μm) ± SD	527 ± 33	536 ± 57	548 ± 43	298 ± 57	341 ± 71	308 ± 45

*$p < 0.05$ versus controls.

Table 3. Hemodynamics and Wall Mechanics in the Iliac and Renal Arteries of Pigs

	C Group		M Group		M+F Group		p-ANOVA
	Iliacs	Renals	Iliacs	Renals	Iliacs	Renals	
Pmax (mm Hg)	142 ± 17	128 ± 22	122 ± 28	125 ± 18	128 ± 23	143 ± 20	0.8
Pmin (mm Hg)	111 ± 10	89.5 ± 35	94.1 ± 23	82.8 ± 23	87.6 ± 23	106 ± 16	0.6
ΔP (mm Hg)	30.0 ± 9.2	28.7 ± 3.5	27.3 ± 11.5	42.3 ± 6.4	40.3 ± 13	36.3 ± 8.1	0.26
Pmean (mm Hg)	123 ± 12	111 ± 15	107 ± 25	100 ± 21	104 ± 22	124 ± 18	0.99
Qmax (ml/min)	575 ± 126	267 ± 108	897 ± 292	415 ± 144	1049 ± 299†	254 ± 59	< 0.0001
Qmin (ml/min)	90 ± 87	177 ± 38	267 ± 143*	210 ± 77	142 ± 111	156 ± 30	0.8
ΔQ (ml/min)	483 ± 92	96.2 ± 31	629 ± 189	171 ± 94	906 ± 424*	97.6 ± 34	< 0.0001
Qmean (ml/min)	234 ± 129	218 ± 54	527 ± 292	306 ± 102	445 ± 273	197 ± 49	0.01
Dmax (cm) × 10^3	584 ± 49	347 ± 7	620 ± 72	540 ± 24†	583 ± 45	550 ± 39†	< 0.0001
Dmin (cm) × 10^3	581 ± 49	335 ± 15	616 ± 69	539 ± 24†	581 ± 43	547 ± 38†	< 0.0001
ΔD (cm) × 10^5	350 ± 66	180 ± 89	400 ± 306	110 ± 12	265 ± 207	273 ± 202	0.02
D mean (cm) × 10^3	581 ± 49	344 ± 9	618 ± 71	540 ± 24†	582 ± 44	544 ± 33†	< 0.0001
Hm (%) × 10^4	166 ± 1	385 ± 222	167 ± 1	284 ± 3	166 ± 1	284 ± 2	0.0002
Co (ml/mm Hg) × 10^3	1166 ± 699	295 ± 112	634 ± 343	304 ± 191	458 ± 225	499 ± 172	0.002
PR (%) × 10^3	622 ± 311	596 ± 162	452 ± 320	183 ± 44*	337 ± 75	390 ± 287	0.3
Zc (mm Hg/ml) × 10^3	89 ± 18	383 ± 58	67 ± 15	190 ± 77†	55 ± 14	418 ± 168‡	< 0.0001
PR (mm Hg/ml) × 10^3	734 ± 174	578 ± 151	281 ± 134†	511 ± 55	381 ± 215†	980 ± 326†‡	< 0.0001
ArS (mm Hg/mm)	1618 ± 628	2471 ± 995	2228 ± 976	3151 ± 401	1910 ± 552	2700 ± 1312	0.004
Ep (mm Hg/mm) × 10^{-2}	467 ± 106	199 ± 66	684 ± 251	656 ± 66†	487 ± 99	472 ± 222	0.05
Sm (kN/m^2)	289 ± 63	85 ± 37	320 ± 74	155 ± 6	272 ± 53	141 ± 35	< 0.0001

*$p < 0.05$ versus controls. †$p < 0.01$ versus controls. ‡$p < 0.01$: M+F significantly different from M group.

Results are mean ± SD for control (C group), hyperhomocysteinemic pigs (M group), and folate-treated hyperhomocysteinemic pigs (M+F group). Pmax indicates systolic blood pressure; Pmin, diastolic blood pressure; ΔP, pulse pressure; Pmean, mean blood pressure; Qmax, systolic blood flow; Qmin, diastolic blood flow; ΔQ, pulse blood flow; Qmean, mean blood flow; Dmax, diastolic external diameter; Dmin, systolic external diameter; ΔD, pulse external diameter; Dmean, mean external diameter; Hm, mean wall thickness–mean radius ration; Co, compliance; Zc, input impedance; PR, peripheral resistance; ArS, arterial wall stiffness; Ep, Young's elastic modulus; Sm, midwall arterial stress. p-ANOVA, p of iliac versus renal comparison according to two-way analysis of variance.

crease in mean flow were observed. In renal arteries, an increase occurred in arterial diameter without significant modification of mean blood flow in the hyperhomocysteinemic animals.

EFFECT OF FOLIC ACID TREATMENT. Iliac arteries of the M+F group showed the same increase in mean flow as in the M group. In the renal bed, there was the same increase in arterial diameter as in the M group; peripheral resistances were higher in M+F group than in the M and C groups. Flow-pressure relationship was not modified by folic acid.

DISCUSSION

The main result of our study is that a treatment with folic acid failed to prevent arterial lesions and thrombotic events in the hyperhomocysteinemic pigs.

Hyperhomocysteinemic pig model. This pig model has been selected rather than another animal model because of two main reasons. First, results of sulfur amino acid metabolism experiments in this species more closely match the results obtained in humans than do those from rabbits and rats (17). Second, we have previously shown that minipigs fed a methionine-rich diet for four months develop hyperhomocysteinemia, thrombotic events and arterial lesions similar to those of homocystinuria (17). In the present study, a four-month methionine-rich diet consistently elicited arterial lesions in all studied vascular beds. The observed lesions in pigs fed a methionine-rich diet were similar to those previously described, as associated hypertrophy of endothelial cells, hyperplasia of smooth muscle cells and major elastic lamina dislocations. Videodensitometric analysis confirmed the decrease in arterial elastin content of hyperhomocysteinemic animals (19). For these analyses, we used a conventional fixation in formol, which can create tissue shrinkage.

However, there was a clear difference at conventional histology and at videodensitometry between controls and hyperhomocysteinemic animals. Using scanning electron microscopy, we have more acutely described endothelial alterations. The endothelial cells were bulging in vascular lumen and in some places were separated by intercellular holes ~2 μm in diameter. However, these lesions may be overestimated because of fixation artifacts. These observations are in agreement with other previous experimental models, either in vivo or in vitro, which usually found morphologic and functional alterations of endothelial cells submitted to high homocysteine concentrations.

Hemodynamic alterations were less apparent in the renal and iliac beds than the alterations previously described in the abdominal aorta (17). Thrombotic events occurred in 4 of the 16 pigs fed a methionine-rich diet; two had myocardial infarction and two had venous thromboembolism. We did not find abnormalities of plasma fibrinogen nor of International Normalized Ratio in the pigs fed a methionine-rich diet. As marked endothelial alterations were found in these pigs, we believe that thrombotic events

in this model are related to thrombosis initiation at the contact of an altered endothelium and thrombogenic subendothelium structures. However, we cannot rule out the possibility of a hemostatic abnormality because we did not perform an exhaustive study of hemostasis. To our knowledge, no abnormalities of hemostatic factors have been found in humans with mild hyperhomocysteinemia.

Effect of a three-month folic acid treatment. Folic acid treatment was begun one month after the onset of the methionine-rich diet to mimic the treatment of hyperhomocysteinemic humans. We verified that one month of this diet was enough to induce a marked hyperhomocysteinemia. Folic acid significantly lowered homocysteinemia. This result is in accordance with previous observations in humans (10,13–15).

Despite a marked lowering in homocysteinemia, folic acid did not prevent hemodynamic alterations, thrombotic events, or arterial lesions. In renal arteries, hemodynamics were similar for both the M and the M+F group. All the pigs of the M + F group displayed arterial lesions similar to the lesions of the M group. We have previously shown that in the media of pigs fed a methionine-rich diet, there is a 30% to 50% decrease of elastin content (19). In this study, folic acid treatment did not modify medial elastin content.

The folic acid dose given in our study was much higher than the Recommended Daily Allowance for humans (200 μg/liter) (14). This 5-mg/day dose has been shown to decrease plasma homocysteine levels of about 25% to 45%. In humans, a lower dose can be efficient. For instance, Ubbink et al. (14) obtained a 41.7% homocysteine reduction with a daily dose of 0.65 mg folic acid for six weeks.

A possible explanation for the absence of effect on arterial lesions in our study could be that a one-month methionine-rich diet induced too much severe lesions to be reversed by a three-month folic acid treatment. However, the extent of arterial lesions one month after the beginning of the methionine-rich diet is not known. Another explanation is that besides homocysteine elevation, an animal-protein-rich diet can induce arterial wall lesions by other mechanisms unknown at the present and unaffected by folic acid treatment (21). A third explanation could be that a concomitant administration of vitamin B-6 and/or vitamin B-12 with folic acid is needed to allow a prevention of arterial lesions. Vitamin B-12 is a cofactor for homocysteine remethylation, and vitamin B-6 stimulates homocysteine transformation into cysteine. Treatments with these vitamins have little effect on fasting homocysteinemia in patients with mild hyperhomocysteinemia (8). The combination of folic acid + vitamin B-6 + vitamin B-12 seems not markedly more effective than folic acid monotherapy for plasma homocysteine reduction (14).

However, the association of folic acid with either vitamin B-6 or vitamin B-12 has theoretical advantages. Vitamin B-6 has an additional effect while reducing homocysteine postprandial peak (21). Vitamin B-12 may be useful in some patients, as hyperhomocysteinemia can be due to vitamin B-12 deficiency and as folic acid will not correct the neuropathy due to this deficiency. We therefore propose, as previously stressed by Ubbink (14), that homocysteine-lowering trials in humans should use combination of folates+vitamin B-6+vitamin B-12.

Reprint requests and correspondence: Dr. Pierre Ambrosi, Department of Cardiology, Hôpital de la Timone, 13385 Marseille, France.

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